IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Pinchera et al.

Serial No.: 10/532,447

Filing Date: April 22, 2005

For: 3,5,3'- TRIIODOTHYRONINE SULFATE AS THYROMIMETIC AGENT AND PHARMACEUTICAL FORMULATIONS THEREOF

Examiner: L. ZHANG

Group Art Unit: 1614

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RULE 132 DECLARATION OF DR. ALDO PINCHERA

Dr. Aldo Pinchera declares and states that:

Background

1. I am a co-inventor of the invention described in the present application (U.S.S.N. 10/532,447) relating to oral compositions of triiodothyronine sulfate ("T_3S"), which is assigned to Bracco SpA ("Bracco") and have reviewed the pending claims.

2. I hold a MD degree from the University "La Sapienza" of Rome. I have published over 547 scientific articles. A copy of my *curriculum vitae* is attached.

3. I am presently Professor of Endocrinology at the Medical School of the University of Pisa; Head First Division of Endocrinology and Metabolism, University Hospital of Pisa; Director, First Postgraduate School of Endocrinology and Metabolic Diseases, University of Pisa; Head, WHO Collaborating Center for the Diagnosis and Treatment of Thyroid Cancer and Other Thyroid Diseases; Regional Coordinator for West Central Europe of the International Council for the Control of Iodine Deficiency Disorders; President University Press of Pisa; and

President, National Committee of Postgraduate Schools of Medical Specialties, Ministry of University and Ministry of Health.

4. I make this declaration to support the patentability of the claims of the present application. Specifically, I make this declaration to submit the details and results of a Phase I clinical trial carried out under my supervision in which thyroidectomized human subjects were administered, once, a dose of oral T_3S compositions (of varying T_3S doses) and gastrointestinal absorption, conversion to the clinically active TT_3 and safety and tolerance were monitored. I am informed that each of the tested oral T_3S compositions was within the pending claims and thus each was an oral composition of the invention.

Summary of the Clinical Trial

5. Twenty eight human subjects with surgically excised thyroids were administered a single dose of 20, 40, 80 or 160 μg of an oral T_3S composition of the invention. The gastrointestinal absorption of T_3S was assessed by serum levels of thyroid hormone including T_3S and triiodothyronine ("T_3") as both free T_3 ("FT_3") and total T_3 ("TT_3"). Safety and tolerance were assessed by monitoring vital signs (blood pressure and heart rate), EEG and hematology, blood chemistry and urinalysis. Subjects without thyroid glands have no endogenous thyroid hormone production; thus the measurement of thyroid hormones levels during the study was not biased by any interference due to the endogenous production. This allowed for measurement of even small changes in the serum concentration of FT_3 and T_3S.

6. All subjects, regardless of dose, exhibited gastrointestinal absorption of the oral composition as shown by detection of T_3S in serum with a peak level two hours after administration of the oral composition. See Figure 1.

7. In patients lacking a thyroid there is no endogenous T_3. Thus, all T_3 present in the subjects was the result of conversion of T_3S from the oral compositions to T_3 in vivo. By monitoring serum T_3S and TT_3 levels after administration of the oral T_3S compositions, it was

determined that T_3S was converted to the clinically active TT_3 in a dose related fashion. See Figure 2.

8. Given the teaching of Lopresti et al, J. of Clin. Endocrinology and Metabolism, Vol 73, No 4, 1992, pages 703-709 ("Lopresti") that radio-labelled T_3S was not found in the serum of patients upon oral administration, these results were unexpected.

Clinical Trial Protocol

Ethical Issues

9. This study was conducted in Pisa, Italy under the guidelines provided in the Declaration of Helsinki, ICH E6 Guideline for Good Clinical Practice, and the requirements of the European Directive 2001/20/EC, and Decreto Legislativo 24 giugno 2003, n. 211 implementing Directive 2001/20/CE in Italy , as well as the European Commission Directive 2005/28/EC of 8 April 2005, laying down principles and detailed guidelines for good clinical practice for investigational medicinal products for human use, as well as the requirements for authorisation of the manufacturing or importation of such products, and related guidance.

10. As T_3S is normally present in the body, the risk involved in administering the selected doses for T_3S was negligible. Indeed, the study design guaranteed that plasma levels of TT_3 could not exceed 196.6 ng/dl, the level obtained by the administration of the consolidated standard therapy of 20 μg T_3. Therefore, no safety concern was foreseen.

Eligibility Criteria and Number of Subjects

11. The criteria for eligibility for the study was as follows:

- Written Informed Consent provided
- Age: between 18 and 70 years
- Gender: either
- Hypothyroidism following complete surgical excision of the thyroid due to thyroid carcinoma, and withdrawal of any substitute T_4 therapy for at least 30 days and T_3 therapy for 24 hours.
- Scheduled for ^{131}I radiotherapy

- Under acceptable metabolic control
- Not suffering from any severe metabolic, organ or systemic disease (excluding the hypothyroid state).

12. The study included a total of 28 subjects. The subjects were divided into 5 groups. Four of the groups were dosage groups of four patients each, which received a dose of an oral composition containing 20, 40, 80 or 160 μg T_3S in tablet form. A fifth group of patients (12 total) received a dose of an oral composition containing 160 μg T_3S in tablet form.

13. This study was not powered based on statistical considerations because this was the first-in-man exploratory and pivotal study. Its goal was to determine if the oral formulation of T_3S was absorbed in the intestinal tract, converted into T_3 and was well tolerated . The number of patients per dose group in the first part of this study was considered sufficient to demonstrate absorption. The 16 patients for the selected dose group was considered sufficient to provide preliminary information about the rate of absorption and the inter-subject variability in the absorption profile.

Methods

14. Forty eight hours prior to administration of the oral T_3S composition of the invention, patients were screened for the study criteria and informed consent was requested and obtained. Twenty-four hours prior to administration of the oral T_3S composition of the invention the subject was examined and all specimens for laboratory tests were collected, including thyroid function tests. On the day of the administration of the oral T_3S composition of the invention, a further check of the inclusion/exclusion criteria was performed and patients were given a single dose of the oral T_3S composition of the invention in tablet form according to the dose group in which they were placed.

15. The tablet composition was as follows:

Tablet composition

Ingredient	Amount per Tablet
T3-Sulphate sodium salt *Equivalent to* T3-Sulphate	20.6 µg 20 µg
Calcium carbonate	30 mg
Glycerol dibehenate	5 mg
Croscarmellose sodium salt	3.5 mg
Hydrate colloidal silica	2 mg
Magnesium stearate	0.5 mg
Microcrystalline cellulose	To 110 mg

16. The tablets of T_3S were presented in bottles containing the individual patient dose as follows:

- First dose-group: 1 tablet in each bottle;
- Second dose-group: 2 tablets in each bottle;
- Third dose-group: 4 tablets in each bottle;
- Fourth dose-group: 8 tablets in each bottle; and
- Fifth (Selected dose) group: 8 tablets in each bottle.

17. In the initial part of the study, the dose groups consisted of 4 patients each. The initial dose was a tablet containing 20 µg T_3S and this dose was increased to the next level (40 µg T_3S) only after 4 patients per dose-group had shown TT_3 plasma levels lower than 196.6 ng/dl. Subsequent increases in dosage likewise occurred only after 4 patients per dose group had shown TT_3 plasma levels lower than 196.6 ng/dl. As none of the 4 patients treated with the 20, 40, 80 and 160 µg doses of the oral T3S composition of the invention had serum levels of TT3 exceeding 196.6 ng/dl, the 160 µg dose was selected for use in the second part of the study.

18. In the second part of the study a fifth group consisting of 12 subjects received a single dose of the oral composition of the invention containing 160 µg T_3S.

19. The absorption of T3S was assessed by measuring the serum levels of thyroid hormones TT_3, FT_3, T_3S, free thyroxine ("FT_4") and Thyrotropin (or Thyroid Stimulating hormone, "TSH"). Additionally all patients were monitored for safety and tolerability.

20. All patients underwent the same observations and evaluations. Thyroid function was measured by:

a) Serum concentrations of TT_3, T_3S and free T_3 FT_3 were determined at the following times:

- 24 hours ±15 min prior to administration,
- at baseline (i.e. within 30±5 min prior to administration),
- after administration at: 1, 2, 4 hours ± 5 min and 8, 12, 24 and 48 hours ± 15 min.

b) Serum levels of TSH and FT_4 were determined at 24 h and 30 minutes prior to administration, and at 24 and 48 hours ± 15 min after the administration of T_3S composition.

21. Gastrointestinal absorption of T_3S was assessed by measurement of circulating serum concentrations of TT_3, T_3S and FT_3. Circulating serum concentrations of FT_3 was measured pre and post dose to verify the in-vivo T_3S - FT_3 conversion in patients.

22. Safety and tolerability were assessed by monitoring adverse events and by monitoring effects on vital signs, ECG , hematology, blood chemistry and urinalysis after administration of the oral compositions of the invention.

Results

23. Regardless of dose, the oral T_3S compositions of the invention were found to be safe and well tolerated both in terms of the adverse event profile and the effects on vital signs, ECG, etc.

24. The mean serum concentration of T_3S (in ng/dl) for each of the four dose groups in the initial part of the study is shown in Figure 1. For each dose group T_3S was present in the serum, with a peak level two hours after oral administration. As all subjects were thyroidectomised and thus lacking endogenous T_3S this data establishes that the T3S from the oral compositions of the invention is absorbed by the gastrointestinal tract and enters the blood.

25. The mean serum concentration of T_3S and TT_3 after administration of a 160μg dose of the oral composition of the invention is shown in Figure 2 for a patient. TT_3 was detected within 4-5 hours of administration of the oral T_3S. As all subjects were thyroidectomised and thus lacking endogenous T_3 this data establishes that once absorbed, the T_3S from the oral compositions of the invention is converted to the clinically active T_3 in a dose-related fashion.

26. These results are unexpected given the Lopresti article, which indicates that oral administration of radio-labelled T3S had no detectable biological activity, was not clinically active and presumably was not absorbed by the gastrointestinal system.

27. I declare that all statements made of my own knowledge are true and that all statements mad eon information and belief are believed to be true, and that these statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of the Title 18 of the United States Code, and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 8/04/2010

Prof. Aldo Pinchera

Figure 1. Mean serum concentrations of T3S (ng/dl) levels in the four groups (native values)

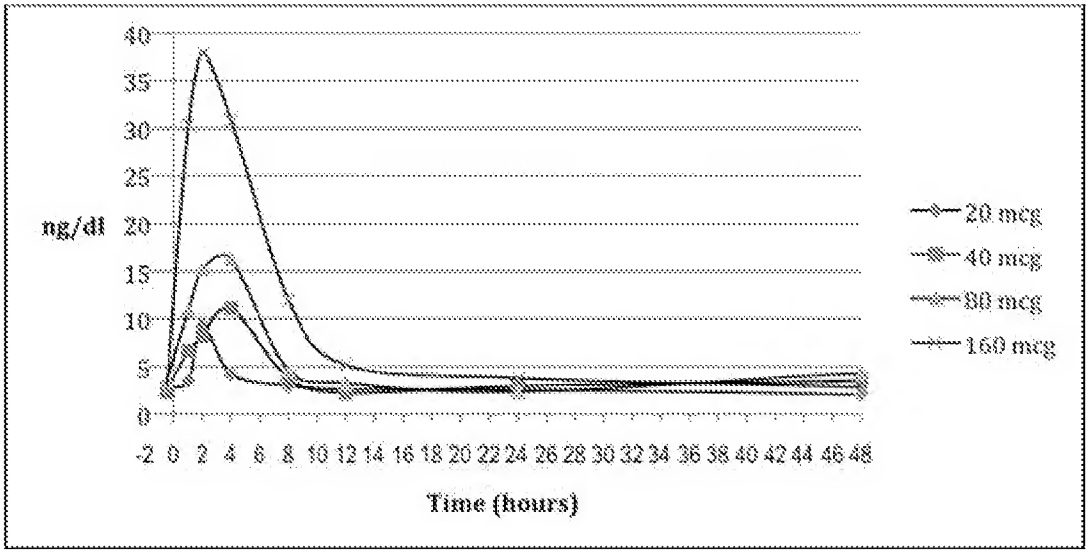

Figure 2: Mean serum concentration curves of T3S and Total T3 after administration at a dose of 160µg